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Biomedical Engineering

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InterLab Study: Colony PCR

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Colony PCR

1 Colony Picking

Estimated bench time: 10 minutes per plate
Estimated total time: 10 minutes per plate
Purpose: Picking single colonies for further processing.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

1.1 Materials

- Autoclaved Eppendorf tubes
- Autoclaved H_2O (nuclease free water)
- Bunsen Burner
- Pipettes and tips
- Plates with the bacterial colonies

1.2 Setup & Protocol

- Fill out Eppendorf tubes with 15 µl autoclaved H_2O.
- Pick bacterial colonies (±5 per plate) near the Bunsen flame with a pipette tip.
- Place the colonies into correct labeled Eppendorf tubes.
- Pipette up and down such that they are mixed well.

2 Colony PCR

Estimated bench time: 30 minutes
Estimated total time: 10 minutes per plate
Purpose: Verifying is the colony contains the correct insert.

When performing a colony PCR, you are working with bacteria close to a Bunsen Burner. Make sure to disinfect your hands. When preparing the MasterMix, make sure to use gloves to protect the MasterMix from DNase activity.

2.1 Materials

- 2X KAPA2G mix
- Autoclaved H_2O (nuclease free water)
- Bucket with ice
- Pair of primers which correspond to correct binding sites on the vector
- PCR tubes
- Pipettes and tips
- The colonies (from colony picking)

2.2 Setup & Protocol

- For one colony PCR reaction, the following mix should be made:

Component	Quantity/mass/final concentration	Volume (µl)
DNA (from bacteria)	Pipette tip in 15 µL H_2O	1
2x KAPA2G mix	1x	12.5
Primer FW	0.5 µM (stock: 10 µM)	1.25
Primer RV	0.5 µM (stock: 10 µM)	1.25
H_2O		9
Total		25

- In order to simplify this step, prepare a MasterMix (2-3 reactions in excess). Keep the mix on ice. Do not add the bacterial DNA but take it into account while calculating the amount of required H_2O.

Component	Quantity/mass/final concentration	Volume (µL)
DNA (from bacteria)	Pipette tip in 15 µL H_2O	1 for each separate PCR mixture
2x KAPA2G mix	1x	
Primer FW	0.5 µM (stock: 10 µM)	
Primer RV	0.5 µM (stock: 10 µM)	
H_2O		
Total		

- Distribute the Mastermix over PCR tubes, thus 24 µl for each tube.
- Add 1 µl of bacterial sample to PCR mixture.
- Run the following PCR program.

Step	Temp °C	Time (sec)	Cycles
Initial denaturation	95	180 (3 min)	1
Denaturation Annealing Extension	95 X[1] 72	15 15 20 sec/kb	35
Final extension	72	600 (10 min)	1
Cooling	4	Hold	1

3 Gel electrophoresis

Estimated bench time: 40 minutes
Estimated total time: 1.5 hours
Purpose: Agarose gel electrophoresis may be used to verify the purity and length of your PCR product. If the product is pure, a single bond will show up during the gel electrophoresis.

[1] The annealing temperature can be calculated for the set of primers using New England Biolabs Tm calculator. An annealing temperature of 3°C lower than the lowest melting temperature was used to increase yields.

3.1 Gel preparation

Estimated bench time: 30 minutes
Estimated total time: 1 hour
Purpose: Preparing a gel on which the samples can be loaded.

It is essential to work with Nitrile gloves when adding SybrSafe.

Select the right percentage agarose gel depending on the DNA size.

Percent agarose gel (w/v)	DNA Size Resolution (kb=1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

3.1.1 Materials

- 1X TAE buffer
- Agarose gel comb
- Agarose gel tray
- Autoclave tape
- Erlenmeyer
- Microwave
- Pipettes and tips
- Purified agarose
- SybrSafe

3.1.2 Setup & Protocol

- Determine how many PCR mixtures will be analyzed. When you plan on analyzing >8 samples, prepare a larger gel (130 ml), when you have ≤8 samples, you can make a smaller gel (65 ml). Pick a gel tray corresponding to the gel you will make.
- Close the sides of the gel tray using autoclave tape. Make sure there are no openings left. Pick the right comb for the gel, i.e. make sure that your gel contains enough trays to load the samples.
- Determine the expected size of the amplicon.
- Weigh the correct amount of agarose for a gel volume of either 65 ml (small gel) or 130 ml (large gel). A 1.0% gel corresponds to 1 gram of agarose in 100 ml.
- Add 1X TAE buffer to the Erlenmeyer with agarose up to the correct volume.
- Weigh the Erlenmeyer containing agarose-TAE.
- Microwave for 1-3 minutes, swirl every 30 seconds (until the agarose is completely dissolved and there is a nice rolling boil).
- Weigh the Erlenmeyer again and add H_2O to correct for the volume loss caused by heating.
- Let the mixture cool down to 50-60°C and add SybrSafe (10.000X stock). Wear nitrile gloves to prevent contact with SybrSafe. Mix well.
- Load the mixture into the gel tray with the comb in it and let it solidify on the bench for approximately 30 minutes.

3.2 Sample preparation

Estimated bench time: 5 minutes start-up + 1 minute per sample
Estimated total time: 5 minutes start-up + 1 minute per sample
Purpose: Loading dye is used to enable visual tracking of DNA migration during electrophoresis. Moreover, loading dye contains glycerol which ensures that the sample forms a layer at the bottom of the well.

Wear gloves, as you are working with DNA.

3.2.1 Materials

- Loading dye
- PCR samples
- Pipettes and tips

3.2.2 Setup & Protocol

- Prepare the samples with 6X loading dye, i.e. add 1 volume of loading dye to 5 volumes of sample. Pipette up and down to mix the loading dye with the sample.

3.3 Loading of the ladder and samples

Estimated bench time: 5 minutes start-up + 1 minute per sample
Estimated total time: 5 minutes start-up + 1 minute per sample
Purpose: Loading the DNA samples within the wells created by the well comb

Wear gloves, as you are working with DNA.

3.3.1 Materials

- 1X TAE buffer
- Agarose gel electrophoresis system
- DNA ladder
- Pipettes and tips
- Prepared samples
- Solidified agarose gel

3.3.2 Setup & Protocol

- Remove the autoclave tape from the solidified gel. Place the gel on the gel tray within the electrophoresis system. Make sure that the comb is located at the negative electrode.
- Add 1X TAE buffer to the gel electrophoresis system until the gel is completely submerged by the TAE buffer.
- Carefully remove the gel comb from the agarose gel.
- Load either the DNA ladder or the samples within the wells. Make sure that the sample or ladder sink to the bottom of the well. Load approximately 300 ng of DNA, i.e. ~5 µl of ladder and ~20 µl of PCR product.

3.4 Running the agarose gel

Estimated bench time: 2 minutes
Estimated total time: 60 minutes
Purpose: Letting the DNA move through the gel.

- Run the gel for approximately 60 minutes at 100V.

3.5 Analysis of the gel using an agarose gel imager

Estimated bench time: 10 minutes
Estimated total time: 10 minutes
Purpose: Taking an image of the gel.

Note: wear gloves while carrying the gel as the TAE buffer may be irritating.

3.5.1 Materials

- Gel to be analysed
- ImageQuant system

3.5.2 Setup & Protocol

- Take out the gel tray from the agarose gel system. Carry the gel tray to the ImageQuant system.
- Turn on the ImageQuant system while it is still closed. Start-up the software while the system is still closed as it may turn on UV light as it boots.
- Turn off the UV light and turn on trans illumination. Place the gel in the ImageQuant system. Zoom in or out using the lens and make sure the image is focused. Close the door and turn off illumination with white light.
- Turn on the UV light and take a picture of the gel. Tweak the exposure time to obtain a better image.
- Turn off UV light, take out the gel and clean the system.